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14. ABSTRACT:
The goal of this project is to determine whether bone mineral density (assumed to be an integrated marker of sex steroid hormone exposure) is a risk factor for prostate cancer; and (2) to identify prostate cancer susceptibility alleles among genes in the sex steroid pathway. To address these aims, we undertook a case-control study of African American and Caucasian men in Pittsburgh, PA and Birmingham, AL. As of 6/31/06, 234 Caucasian and 56 African American (AA) cases and 237 Caucasian and 67 AA controls with PSA <3.0 ng/mL frequency matched by age and race to Hip, spine and total body BMD is measured by Dual-energy X-ray Absorptiometry (DXA) on all particpants. Blood specimens have been used to isolate DNA on all subjects. Polymerase Chain Reaction (PCR) techniques were used to determine allelic distributions of genotypes for sex steroid metabolism, biosynthesis and action genes. Risk factor data were obtained by an in-person interview and are immediately scanned into the study database. All data collection is complete and we are now analyzing the data. We will evaluate the role of BMD and candidate genotypes in prostate cancer risk by race. We will further examine the interaction between BMD and genotypes to evaluate the hormonal environment – gene interaction and its effect on prostate cancer risk.

15. SUBJECT TERMS
prevention, epidemiology, genetic epidemiology, etiology, gene-gene and gene-environment interaction, genetic risk, bone mineral density, sex steroid hormone

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Table of Contents

INTRODUCTION:

The goal of this project was to determine whether bone mineral density (assumed to be an integrated marker of sex steroid hormone exposure) is a risk factor for prostate cancer; and (2) to identify prostate cancer susceptibility alleles among genes in the sex steroid pathway. To address these aims, we undertook a case-control study of African American and Caucasian men in Pittsburgh, PA and Birmingham Alabama. Cases are African American and Caucasian men with histologically-confirmed prostate cancer. Controls are age and race frequency-matched men who have a prostate specific antigent (PSA) level < 3.0 ng/mL. Hip, spine and total body body mass index (BMD) was measured by Dual-energy X-ray Absorptiometry (DXA). Blood was used to obtain DNA. Polymerase Chain Reaction (PCR) techniques will be used to determine allelic distributions of genotypes for sex steroid metabolism, biosynthesis and action genes. Risk factor data were obtained by an in-person interview. Pathology information was collected using standardized medical abstraction. We will evaluate the role of BMD and candidate genotypes in prostate cancer risk by race. We will further examine the interaction between BMD and genotypes to evaluate the hormonal environment – gene interaction and its effect on prostate cancer risk.

BODY:

In this section, we describe our accomplishments according to the Work Plan originally approved:

Recruiting of Subjects and Obtaining of Data

Please see tables 1-3 for summaries for study recruitment. In short, we have recruited 234 Caucasian cases, 237 Caucasian controls, 56 African American (AA) cases and 67 AA controls.

Demographic and risk factor data, including BMD measurements, were obtained on all subjects during an in-person interview.

All data were entered into a database, verified and cleaned. We have randomly reviewed 10% of the interview forms and compared the data with the database to ensure accuracy.

Performance of Laboratory Assays

DNA was isolated on all subjects was quantitated and diluted to 40ng/ul

Laboratory assays to detect sex steroid related genetic polymorphisms were performed on all subjects. These included:

a. CYP17A1-01 rs743572
b. SRD5A2 rs523349
c. CYP19 TTAA Intron 4 repeat
d. Androgen receptor CAG repeat
e. Androgen Receptor GGC repeat
f. SHBG TAAAA 5' repeat
g. ESR1 XbaI
h. ESR1 Pvu II

In addition, the following genotyping assays were performed on a subset of specimens:

a. AIB1/SRC3 - steroid receptor coactivator 3; CAG (glutamine) repeat polymorphism

b. CYP11A - cholesterol side chain cleavage enzyme; pentanucleotide repeat

[(TTTTA)n] in the promoter
c. SHBG - pentanucleotide repeat [(TAAAA)n] located in an Alu sequence at the 5' boundary of the promoter
d. CYP19 - aromatase; intronic tetranucleotide repeat [(TTTA)n]
e. HSD11B1 - 11-beta hydroxsteroid dehydrogenase; a CA repeat

Interim Analyses of Data
Tables 1-3: a preliminary review of the demographic and BMD data suggest that the AA population is less likely to be married and have a lower level of education. This most likely reflects the referral patterns of AA with prostate cancer and the practice from which we recruit our Caucasian subjects (Dr. Joel Nelson, Chair of Urology, University of Pittsburgh Medical Center). These differences will be controlled for in our analyses (by including a variable in our models).

Table 4 shows the preliminary review of the genetic data. Initial analyses suggest that the 5SDR SNP may be a potential prostate cancer risk factor. Moreover, the ER SNPs appear to differentially affect Caucasians and African Americans. We are cautious to note that these data are preliminary.

Overall Study Progress:
We completed Caucasian recruitment, but failed to meet our recruitment goals for African Americans for many reasons and despite extensive efforts on our part. We were unable to obtain IRB approval from the DOD Human Subjects Committee for the Baltimore site, the original minority set. We have therefore had to dropped this site from the study. We invested a great deal of time and effort in trying to launch this study in Baltimore, and are disappointed to not include them in the study because in the preliminary recruitment efforts, approximately 2-3 AA cases *per week* were being referred into the study.

We then arranged for a second minority site, Alabama. Despite extensive personnel efforts, recruitment in Alabama was slow. We received DOD IRB approval in 2004 to commence minority recruitment at the Alabama site. The site is under the supervision of Dr. James Shikany. During 2004 we worked with the Alabama investigators to revise the Manual of Operations for their site and to put into place all the study procedures, including sending data and specimens to Pittsburgh. We also trained the field interviewer to ensure consistency in questionnaire administration. Concurrently, the Alabama investigative team met with urologists in the area to set up recruitment procedures. Dr. Shikany and his staff set up recruitment in several urology clinics in the Birmingham area, including Dr. Urban at the University of Alabama, Dr. Tully (AL Urology Associates) and Dr. Cohn (St. Vincent's Medical Center). They also arranged for recruitment at the local VA hospital. Controls have been recruited from healthy men participating at the PLCO trial to ensure compatibility with the Caucasian controls. Despite extensive efforts on the part of Dr. Shikany to recruit AA cases, he was unable to meet his expected goal of 100 cases. Unlike Caucasian recruitment in Pittsburgh, in which the collaborating physicians refer men into the study, the physicians in Alabama required our recruiter to be present in clinic to talk to gentlemen after their doctor visit. This was extremely labor intensive and reduced the number of potential subjects our recruiter can contact per day. In addition, minority men are more reluctant to participate in a study and the no-show rate for AA subjects was high relative to our Caucasian subjects.

We worked with the site PI to provide additional resources and mechanisms to increase enrollment, including extending the study to the local VA hospital. Despite these efforts, Dr. Shikany's recruitment reached only 42 cases over approximately 18 months.

In Pittsburgh, we tried a variety of mechanisms to increase minority recruitment, which for the most part had a minimal effect on recruitment. We worked with the Center for Minority Health to design targeted recruitment materials. We have also worked out a recruitment method with our IRB so that every AA man diagnosed with prostate cancer at a UPMC facility (about 40 men per year) will be informed about the study via a direct mailing and follow-up phone call. These targeted efforts resulted in few enrollees.

Other approaches we tried: we gave presentations at urology meetings and prostate cancer support group meetings throughout the city. We worked with the American Cancer Society and other local organizations to advertise our study and support recruitment. Most African American men with prostate cancer are seen outside the UPMC health system. We have contacted physicians in the other health system to engage their support of our study, and offered to compensate their staff for time engaged in helping with this study (using monies from Dr. Modugno's R&D fund). The two practices that see most African Americans cases in the area refused to cooperate because they do not wish to collaborate with UPMC investigators. We therefore have exhausted all possibilities for African American recruitment in Pittsburgh.

Exclusion Criteria

The following are the criteria used to exclude men from participation in this study.

- <40 or >80 years of age
- Inability to consent to medical procedures.
- History of hyper or hypothyroidism, hyperparathyroidism, renal disease, or bone disorders
- History of hypogonadism
- History of Bone Disease/problems – osteoporosis, Paget's disease, osteomalacia, osteogenesis imperfecta,
- Chronic (>3 months) glucocorticoid therapy
- Use of testosterone supplementation (>3 months)
- Use of bisphosphonate supplementation (>3 months)
- Bilateral hip replacement
- Kidney or liver transplant recipient
- Previous diagnosis of cancer, except basal/squamous cell skin cancer
- trouble absorbing vit D., vit D deficiency, calcium abnormality, brittle bones
- 2 or more non-traumatic fractures over a lifetime or 1 or more non-traumatic fracture in the last year.
- For prostate cancer cases, evidence of bone metastases
- For controls, PSA levels above 3 ng/mL within the last 3 months

Data Collection

The following data are collected on all participants:

- demographics, lifestyle factors and medical history via a ½ hours in-person interview
- hip, spine and total BMD via a DXA scan. Results are abstracted onto a study form
- urine sample (Pittsburgh only)

- 35 ml of blood. This is used to isolate DNA for the current study. In addition, the following specimens are banked:
    - serum (8x1mL)
    - plasma (8x1mL)
    - buffy coat
    - clot
- height, weight and hip circumference (measured by study personnel during study visit). Results are recorded on a study form.

Laboratory Assays

All genotyping assays are done in the laboratory of Dr. Robert Ferrell. High molecular weight DNA is extracted from peripheral blood leukocytes by the salting-out procedure. Polymerase Chain Reaction (PCR) and Restriction Fragment Length Polymorphism(RFLP) techniques will be used to identify polymorphisms in the sex steroid metabolism pathway. Restriction fragment length polymorphisms are genotyped by amplification of the variable site using unique sequence flanking primers, digestion with an appropriate restriction endonuclease, resolution of the fragments on 2% agarose gels and visualization under ultra violet light after ethidium bromide staining. Single nucleotide polymorphisms that do not alter a restriction site are assayed by a modified allele specific oligonucleotide ligation assay. Length polymorphisms are genotyped by amplification using unique sequence flanking primers, one of which is labeled with a fluorescent dye (FAM, HEX or TET; Research genetics, Huntsville, AL). The products are resolved on the ABI 377 automated DNA sequencer (Applied Biosystems, Foster City, CA) and the resulting gel images are analyzed using the GENESCAN software package. These protocols are standard in Dr. Ferrell's lab. Genotypes are assigned by two independent readers by directly comparing test samples to sequence-verified control samples run on the same gel. Conflicts are resolved by repeat genotyping.

We tested the laboratory assays on a sample of specimens early in our recruitment and completed all assays on the subjects.

B**MD Measurements**

Hip, spine and total body BMD will be measured by Dual-energy X-ray Absorptiometry (DXA) using a Hologic QDR-4500A (Hologic, Inc.,Waltham, MA) in the Laboratory of Dr. Susan Greenspan. Quality control is assessed by daily quality control scans with the phantom provided by the manufacturer. All DXA results are recorded on a standard study form for data entry.

Problems encountered and measures taken:

Minority recruitment was very difficult. As discussed above, we took several measures in both Pittsburgh and Alabama to try and increase enrollment. Our experience suggests that even among experienced recruiters of minority populations (such as in Alabama the PLCO and WHI minority recruitment sites), recruitment of minorities is difficult and we would not undertake such a project without extensive staff support.

KEY RESEARCH ACCOMPLISHMENTS:

- completed Caucasian recruitment surpassing recruitment goals in the allotted time
- cleaned all demographic and BMD data

- isolated and quantitated DNA from all samples
- all genotyping assays are completed
- beginning analyses of data

REPORTABLE OUTCOMES:

1. Publications:

*Benjamin Davies**, Joseph Chen, **Francesmary Modugno**, Joel Weissfeld, Doug Landsittel, Rajiv Dhir, Joel Nelson, and Robert H. Getzenberg. Contribution of the Prostate Limits the Utility of Survivin in the Detection of Bladder Cancer. *Journal of Urology* 174(5):1767-70, 2005.

*Claudia C. Leiras**, **Francesmary Modugno**, Joel Weissfeld, and Joel Nelson. Male Pattern Baldness as a Biomarker of Prostate Cancer Risk. In Proceedings *of the American Association for Cancer Research Annual Meeting 2004.* Orlando, FL. March 2004.

2. Specimen Banks
    a. Urine bank
    b. DNA bank
    c. Serum and Plasma Bank

3. Grants applied for and received:
    a. NIH 1U01CA117452

4. Future work
    a. publication of study results in full
    b. application for grants extending any findings
    c. applications for grants using existing specimen and data bank

CONCLUSIONS:

Our initial conclusions suggest no differences in BMD between cases and controls nor between races. However, potential differences in sex steroid genotypes between Caucasians and African Americans may exist. Further analyses will help clarify these findings.

REFERENCES:

None

List of Personnel:

Francesmary Modugno
Doug Potter
Susan Greenspan
Gail Engleka
Chandra Marriott
Betty Kotowski
Pam Overberger
Glenn O. Allen

Table 1: Summary of Recruitment through 6/31/06

	Caucasian		**African-American**	
Age Range	Cases	Controls	Cases	Controls
40-44	3	3	1	5
45-49	4	4	4	3
50-54	23	25	7	12
55-59	65	64	15	7
60-64	72	71	14	11
65-69	46	49	5	20
70-74	18	16	8	6
75-79	3	5	2	3
Total	234	237	56	67

Table 2: Summary Demographic Statistics on Cases through 6/31/06

	Cases		UPMC Cases		UAB Cases		Caucasian Cases		AA Cases	
	n=290	%	n=245	%	n=42	%	n=234	%	n=56	%
Age (yrs) mean	60.69		60.42		62.24		60.81		60.18	
Race										
Caucasian	234	80.7	222	90.6	12	28.6				
African-American	56	19.3	23	9.4	30	71.4				
Recruitment Site										
UPMC	245	84.5					222	94.9	23	41.1
PGH VA	3	1.0							3	5.4
UAB	42	14.5					12	5.1	30	53.6
Education										
<8 yrs	3	1.0			3	7.1			3	5.4
8 to 11 yrs	9	3.1	4	1.6	5	11.9	4	1.7	5	8.9
12 yrs or HS	56	19.3	46	18.8	9	21.4	40	17.1	16	28.6
post secondary	13	4.5	11	4.5	2	4.8	10	4.3	3	5.4
some college	42	14.5	33	13.5	7	16.7	27	11.5	15	26.8
college grad	73	25.2	61	24.9	12	28.6	63	26.9	10	17.9
postgraduate	94	32.4	90	36.7	4	9.5	90	38.5	4	7.1
Marital Status										
never married	11	3.8	9	3.7	2	4.8	6	2.6	5	8.9
married	251	86.6	215	87.8	35	83.3	211	90.2	40	71.4
widowed	6	2.1	4	1.6	2	4.8	4	1.7	2	3.6
divorced	15	5.2	11	4.5	2	4.8	9	3.8	6	10.7
separated	7	2.4	6	2.4	1	2.4	4	1.7	3	5.4
BMI (kg/m2) mean	28.37		28.23		29.23		28.18		29.20	
	n	**Mean**	**n**	**Mean**	**n**	**Mean**	**n**	**Mean**	**n**	**Mean**
BMD (g/cm2)										
Hip	289	1.016	244	1.012	42	1.044	233	1.007	56	1.052
Spine Lateral	159	0.792	156	0.794			141	0.787	18	0.832
Spine PA	288	1.082	243	1.075	42	1.125	232	1.076	56	1.104
Total Body	281	1.171	237	1.155	41	1.267	226	1.157	55	1.228
LBM	281	62.04	237	62.01	41	62.76	226	61.65	55	63.65
% Body Fat	281	25.50	237	25.74	41	23.93	226	25.80	55	24.29

Table 3: Summary Demographic Statistics on Controls through 6/31/06

	Controls		UPMC Controls		UAB Controls		Caucasian Controls		AA Controls	
	n=305	%	n=254	%	n=44	%	n=237	%	n=67	%
Age (yrs) mean	61.20		61.09		64.23		61.29		60.82	
Race										
Caucasian	237	77.7	224	88.2	13	29.5				
African-American	68	22.3	30	11.8	31	70.5				
Recruitment Site										
UPMC	254	83.3					224	94.5	29	43.3
PGH VA	7	2.3							7	10.4
UAB	44	14.4					13	5.5	31	46.3
Education										
<8 yrs	3	1.0	1	0.4	2	4.5	1	0.4	2	3.0
8 to 11 yrs	11	3.6	5	2.0	6	13.6	4	1.7	7	10.4
12 yrs or HS	53	17.4	45	17.7	6	13.6	39	16.5	14	20.9
post secondary	20	6.6	15	5.9	3	6.8	14	5.9	6	9.0
some college	67	22.0	54	21.3	11	25.0	49	20.7	18	26.9
college grad	63	20.7	53	20.9	9	20.5	50	21.1	12	17.9
postgraduate	88	28.9	81	31.9	7	15.9	80	33.8	8	11.9
Marital Status										
never married	22	7.2	18	7.1	2	4.5	16	6.8	6	9.0
married	221	72.5	185	72.8	32	72.7	178	75.1	42	62.7
widowed	15	4.9	12	4.7	3	6.8	12	5.1	3	4.5
divorced	38	12.5	32	12.6	5	11.4	26	11.0	12	17.9
separated	8	3.0	7	2.8	2	4.5	5	2.1	4	6.0
BMI (kg/m2) mean	28.98		29.03		28.2		29.05		28.72	
	n	**Mean**	**n**	**Mean**	**n**	**Mean**	**n**	**Mean**	**n**	**Mean**
BMD (g/cm2)										
Hip	305	1.034	254	1.026	44	1.066	237	1.028	67	1.052
Spine Lateral	113	0.780	106	0.794			105	0.794	8	0.876
Spine PA	305	1.094	254	1.089	44	1.127	237	1.089	67	1.114
Total Body	302	1.181	251	1.166	44	1.262	235	1.171	66	1.216
LBM	302	85.63	251	90.31	44	61.87	235	92.16	66	62.63
% Body Fat	302	25.70	251	25.83	44	24.58	235	25.86	66	25.13

Table 4: Summary SNP Analysis through 6/31/06

		All Subjects				Caucasian only				African American only			
		Controls	Cases	OR	95%CI	Controls	Cases	OR	95%CI	Controls	Cases	OR	95%CI
CYP17A1-01	CC	43	45	1.00		37	42	1.00		6	3	1.00	
C(+27)T	CT	111	129	1.11	0.68 - 1.81	87	101	1.02	0.60 - 1.73	23	28	2.44	0.55 - 10.82
rs743572	TT	115	89	0.74	0.45 - 1.22	84	71	0.75	0.43 - 1.28	31	18	1.16	0.26 - 5.22
CYP17A1-01	TT	115	89	1.00		84	71	1.00		31	18	1.00	
C(+27)T	CT	111	129	1.50	1.03 - 2.19	87	101	1.37	0.90 - 2.10	23	28	2.10	0.94 - 4.67
rs743572	CC	43	45	1.35	0.82 - 2.23	37	42	1.34	0.78 - 2.31	6	3	0.86	0.19 - 3.87
SRD5A2	GG	151	139	1.00		119	113	1.00		31	26	1.00	
V89L G/C	GC	102	105	1.12	0.78 - 1.60	74	83	1.18	0.79 - 1.77	28	22	0.94	0.44 - 2.01
rs523349	CC	16	19	1.29	0.64 - 2.61	15	18	1.26	0.61 - 2.63	1	1	1.19	0.07 - 20.01
ESR1 XbaI	AA	90	103	1.00		65	85	1.00		24	18	1.00	
	AG	127	116	0.80	0.55 - 1.17	100	89	0.68	0.44 - 1.05	27	27	1.33	0.59 - 3.00
	GG	39	28	0.63	0.36 - 1.10	36	26	0.55	0.30 - 1.01	3	2	0.89	0.13 - 5.89
ESR1 PvuII	CC	68	63	1.00		50	44	1.00		18	19	1.00	
	CT	136	116	0.92	0.60 - 1.41	111	94	0.96	0.59 - 1.57	24	22	0.87	0.37 - 2.07
	TT	52	69	1.43	0.87 - 2.35	40	63	1.79	1.02 - 3.15	12	6	0.47	0.15 - 1.53

Length Polymorphisms	Control Mean	Case Mean	p-value	Control Mean	Case Mean	p-value	Control Mean	Case Mean	p-value
AR CAG	274.52	274.51	0.995	275.93	275.76	0.848	269.28	269.08	0.917
AR GGC	194.61	195.17	0.225	195.32	195.30	0.969	192.16	194.55	0.033
CYP19 Allele 1	359.36	359.11	0.668	359.60	359.42	0.794	358.53	357.67	0.367
CYP19 Allele 2	370.33	370.71	0.611	370.89	371.32	0.587	368.19	367.77	0.834
SHBG Allele 1	165.72	165.69	0.963	166.04	165.85	0.707	164.66	165.00	0.737
SHBG Allele 2	173.91	174.03	0.793	174.25	174.12	0.793	172.80	173.55	0.511